

Supplementary Table S1 | Mutations detected in tigecycline/tobramycin evolved *P. aeruginosa* PA14 populations at the end of the evolution.

Gene	Replicate	Mutation	Nucleotide localization	Aa Change	Coverage* (%)
BOTH TREATMENTS					
<i>orfN</i>	4, 8	Ins G	138-139	Val50fs	80/61,64
	1, 2, 3, 5, 6, 7	Del G	139	Val50fs	68,90/70,98/ 87,41/84,84/ 83,68/85,55
TIGECYCLINE TREATMENT					
<i>PA14_00180</i>	5	C-->A	146	Arg49Leu	96,68
	8	C-->T	752	Cys251Tyr	100
<i>rpsj</i>	5, 8	G-->C	169	Val57Leu	100/98,97
<i>parR</i>	5	G-->A	259	Glu87Lys	98,51
	6	G-->A	640	Glu214Lys	98,36
<i>secA</i>	5	G-->A	1475	Ala492Val	98,41
<i>mexD</i>	5	A-->C	1823	Phe608Cys	99,51
<i>nfxB</i>	5	T-->C	452	Leu151Pro	98,04
	6	Del CTGAAAGA ACT	76	Leu26fs	53
	7	Del GGAGGC	231-236	Glu78_Ala79	82,85
<i>frr</i>	7	Ins GGAGGC	394-395	Lys132delinsArg ArgGln	98
	8	Del C	494	Ser166fs	92,85
	6	G-->A	3	Met1Ile	100
<i>mexC</i>	6	C-->A	311	Ser104*	84,46

<i>secG</i>	6	Del A	194	Phe65fs	93,21
<i>rpoN</i>	7	G-->C	64	Ala22Pro	100
<i>parS</i>	8	C-->G	553	Arg185Gly	100
TOBRAMYCIN TREATMENT					
<i>fleQ</i>	2	T-->G	721	Thr241Pro	53,19
<i>ptsP</i>	3	Del G	2156	Glu677fs	97,73
	2	G-->T	2029	Asp720fs	99
	1	A-->G	2011	Thr671Ala	99,42
<i>fusA</i>	2	G-->C	1783	Ala595Pro	100
	3	G-->A	1634	Gly545Asp	100
	4	C-->T	2038	Arg680Cys	99,31
INTERGENIC MUTATIONS					
UPS: TrmH family RNA methyltransferase	1, 3, 5, 7, 8	Del C	5809306	-	84,75/83,99/ 84,11/83,75/
DNS: <i>rpsF</i>					79,27
UPS: hypothetical protein	2	G-->T	5556124	-	44,38
DNS: cAMP binding protein A					
UPS: <i>tyrZ</i>					
DNS: 16S ribosomal RNA	6	GC-->AT	732714..732 715	-	89,29
UPS: <i>tyrZ</i>					
DNS: 16S ribosomal RNA	6	T-->C	732732	-	92,31

The Table shows the nucleotide location of the mutations and associated amino acid changes. Genetic modifications in intergenic regions are also included, and their locations refer to the nucleotide position in *P. aeruginosa* UCBPP-PA14 reference chromosome (NC_008463.1). Fs: frameshift. UPS: upstream gene. DNS: downstream gene. Del: deletion. Ins: insertion.

* Coverage represent the percentage of reads of each mutant allele among the total number of reads, corresponding the same region in the genome, within the whole population, at the end of the experimental evolution.

Supplementary Table S2 | Primers used to verify nucleotide modification detected in whole-genome sequencing and to perform real-time RT-PCR.

Gene	Mutation	Localization	Primer Fw (5'-3')	Primer Rv (5'-3')
WGS VERIFICATION PRIMERS				
<i>orfN</i>	Ins G	138-139		
	Del G	139	ATGGACGTTCCCAATGC CCG	CCGCCAGAATCAGCAAA ACC
<i>pmrB</i>	C-->A	772		
	G-->C	853	GCCGAACGCCGACTGAC CAG	AATTGCTCCAGCAGGGC GTC
<i>PA14_00180</i>	T-->G	22		
	T-->C	110	CCTGAAAACCGCCTACC GGA	TTCGGTGGCAAGGTCGA GCA
	C-->T	28		
<i>rpsj</i>	G-->T	583	GTTGCTCGGCGGCCTGG TCT	TGAGTTCGTCGATGAGG CCG
	C-->A	146	TTGCCGCCGCAACTGGA CAA	AACGGCTTCTGCAACAG GCG
<i>parR</i>	C-->T	752	CAACTGGCCGCCGAGCT GCT	CCGCTGGAGGTTCCTCCC GAA
	G-->A	259	CGCGAACCTGCCGATCC TCATA	GCTCGATCGGCTTGATC ACG
<i>secA</i>	G-->A	640	TCGACGTCTGCATCAGC AAG	AACAGGTAGCCCTTGCC CCA
	G-->A	1475	GAGGCAGGCATCGAGCA CAA	ATGTTGGTGGCGATGGT CAC
<i>mexD</i>	A-->C	1823	GAACTCGAGCGCTTCCT CAA	CAGTCCTGAAGGTCGG GAA
<i>nfxB</i>	T-->C	452		
	Ins GGAGGC	394-395	TCCTACCTGGAAGCGCT GGA	CATCTGCTCCAGGGTAT GCG
<i>frr</i>	Del CTGAAA	76	CTCATCAAGGCGCTGGC AGT	ATCTGCACCAAGGTTGTC CCG
	GAACT			
<i>mexC</i>	Del GGAGGC	231-236	GAACCAGATCATCCAGG CCT	CGGTGGGTGAGGTGTT CTT
	G-->A	3	TCTTGCCCCATGCGCTCCT GC	TCTTGCCCCATGCGCTCCT GC
<i>secG</i>	C-->A	311	TTCCAGATCGATCCGGC ACC	CTGCGCCTCGAACAGCA CCG
<i>rpoN</i>	Del A	194	GCTACCTTTTGAGTCGG ATT	TCAGACTTTCTTAGCG AA
<i>pars</i>	G-->C	64	CGCTAGTCCTCAAGATG GGC	ATTCCTGCTGGAGGTC CAG
	C-->G	553	ACCGTGCTGGCCTACAT CCT	TCGAAGGACAGGCGGG AGAT

<i>fleQ</i>	T-->G	721	CTTCACCGGTGCCATCA CCA	AGCAGGGCGATGTCTTC CAC
<i>ptsP</i>	Del G	2156	TGCTGCATGCGTTGAAG	AGCGAGCTGTGGATGAC
	G-->T	2029	AAG	CTG
<i>fusA</i>	A-->G	2011	TATTCGTGCCGAGGTTC	GGAGCTTCGGCGTATT
	G-->T	2038	CGC	GGA
<i>rplU</i>	G-->C	1783		
	G-->A	1634	GGACGAGAAGGGCAAC	CATGATCGGCTCGAGCA
<i>mexC</i>	T-->C	457	ATCA	CCT
	REAL-TIME RT-PCR PRIMERS			
<i>rplU</i>	-	-	CGCAGTGATTGTTACCG GTG	AGGCCTGAATGCCGGTG ATC
<i>mexC</i>	-	-	GACCTGCTGTTCCAGAT CG	AGGACTTCGATAACGCC AC

Supplementary Table S3 | MIC values in the population replicates during selective pressure from tigecycline and tobramycin.

Treatment	Replicate	5 d MIC	10 d 2MIC	15 d 4MIC	20 d 8MIC	25d 16MIC	30 d 32MIC	35 d 32MIC
TOBRAMYCIN	1	6	6	12	16	24	24	24
	2	4	4	8	8	24	24	24
	3	4	4	6	12	16	32	32
	4	4	6	12	16	24	32	32
TIGECYCLINE	5	32	32/≥256	96/≥256	≥256	≥256	≥256	≥256
	6	48	24/≥256	64/≥256	64/≥256	≥256	≥256	≥256
	7	48	32/≥256	64/≥256	96/≥256	≥256	≥256	≥256
	8	32	24/≥256	64/≥256	96/≥256	≥256	≥256	≥256
CONTROLS (no antibiotic)	9	1-2	1-2	1.5-2	1-2	1-2	1-2	1.5-2
	10	1-2	1-2	1.5-2	1-2	1-2	1.5-2	1.5-2
	11	1-2	1.5-2	1.5-2	1.5-2	1-2	1.5-2	1.5-2
	12	1.5-2	1-2	1.5-2	1.5-2	1-2	1.5-2	1.5-2

The table shows the E-test MIC value for each replicate population every five days (the antibiotic concentration was doubled every 5 days). The MICs for the tobramycin and tigecycline controls are indicated as two different values separated by a dash (X-X). Double inhibition halos detected in mixed populations are shown as two MIC values separated by a slash (X/X).

Supplementary Table S4 | Disc diffusion assays of antibiotics of different structural families in the populations evolved at 32MIC tobramycin and tigecycline

Replicate	Tgc	Tet	Imi	Atm	Caz	Cip	Tob	S	Ak	Cs	PB	C	E	F
PA14	11	20	26	24	23	34	21	14	21	17	14	11	8	15
TOBRAMYCIN														
1	0	7/16	28	25	19	27	7	0	0	17	15	14	0	25
2	0	8/19	27	25	20	31	7	0	7	16	14	15	6	26
3	0	9/18	28	23	20	33	13	7	12	17	14	14	8	24
4	0	8/17	24	22	20	27	7	0	0	16	14	15	0	26
TIGECYCLINE														
5	0	0	26	17	18	26	16	6	15	18	14	8	6	24
6	0	0	26	19	15	26	18	7	19	18	14	8	6	24
7	0	0	27	20	18	30	19	6	18	16	14	8	6	26
8	0	0	24	20	20	25	14	0	14	17	14	8	6	18
CONTROLS														
9	10	18	27	26	21	32	19	12	21	17	13	11	7	16
10	12	19	26	26	23	33	19	13	23	17	13	12	8	15
11	12	20	27	25	22	33	19	14	21	17	13	13	8	17
12	10	19	27	25	24	34	20	13	22	17	14	11	7	15

The table shows the diameter of halo (mm) in the disc diffusion assays for each replicate population at the end of the experimental evolution. The antibiotics in which halos differ were selected for an E-test assay (Table 1). Double inhibition halos detected in mixed populations are shown as two values separated by a slash (X/X). Tgc: tigecycline, tet: tetracycline, atm: aztreonam, caz: ceftazidime, cip: ciprofloxacin, tob: tobramycin, s: streptomycin, ak: amikacin, cs: colistin, PB: polymyxin B, c: chloramphenicol, e: erythromycin, f: fosfomycin.